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BRIEF COMMUNICATION

Effects of Subdiaphragmatic Vagotomy on the Acquisition of a Radiation-Induced Conditioned Taste Aversion

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HUNT, W. A., B. M. RABIN AND J. LEE. *Effects of subdiaphragmatic vagotomy on the acquisition of a radiation-induced conditioned taste aversion.* NEUROTOXICOL TERATOL 9(1) 75–77, 1987.—The effect of subdiaphragmatic vagotomy on the acquisition of a radiation-induced taste aversion was examined to assess the importance of the vagus nerve in transmitting information on the peripheral toxicity of radiation to the brain. Vagotomy had no effect on taste aversion learning, consistent with reports using other toxins. The data support the involvement of a blood-borne factor in the acquisition of taste aversion induced by ionizing radiation. Keywords: Ionizing radiation; Conditioned taste aversion; Vagotomy; Nerve transmission; reprints

ONE of the presumed mechanisms by which animals avoid accidental poisoning is through the conditioned taste aversion (CTA). In the wild, animals learn to avoid poisoned food or water by associating the ingested substance and some reaction occurring thereafter. In a laboratory setting, a CTA can be acquired in a single trial after a toxin is paired with the consumption of a novel tasting solution that the animals subsequently avoid [3].

Accumulating evidence suggests that an important area of the brain medating CTAs acquired after exposure to a variety of toxins is the area postrema located in the brain stem. These toxins include, for example, lithium chloride, histamine, copper sulfate, cis-platin, WR-2721, and ionizing radiation. Ablation of the area postrema blocks the acquisition of a CTA induced by all of these toxins ([12, 13, 15–17]; Rabin and Hunt, unpublished observations). Presumably, the toxins do not directly interact with the area postrema for the following reasons. No receptors for the toxins would generally be expected, since these toxins are mostly foreign substances and an organism would not have evolved such receptors. In fact, when lithium chloride injected into the cerebral ventricles is used as the unconditioned stimulus in the CTA paradigm, no aversion is observed, compared to that found after an intraperitoneal injection of the substance [19]. In the case of ionizing radiation, exposure of the head produces significantly less of an aversion than exposure to the body alone [2, 14, 20], especially to the abdomen [2].

There are two general mechanisms by which toxins could interact with the area postrema. One mechanism that has been postulated is the release of a humoral factor from affected tissues into the blood after exposure to the toxin [4]. This factor presumably would interact with the area postrema or through some intermediate mechanism. The hypothesis of a humoral factor is based on experiments with parabiotic rats, where one animal was exposed to ionizing radiation, while the other animal was shielded with lead [4]. A CTA developed in both animals.

Other possible mechanisms involving neural feedback to the area postrema are also possible. Since many toxins can irritate or damage the gastrointestinal system, information on this damage might be transmitted to the area postrema over neural pathways originating in the gastrointestinal system. Anatomical studies have demonstrated that the area postrema receives input from the vagus nerve [8,18] (the major afferent input to the brain stem from the gastrointestinal system [10]). Consequently, lesions of the area postrema could disrupt both a humoral and a neural mechanism underlying the acquisition of learned taste aversions. In an attempt

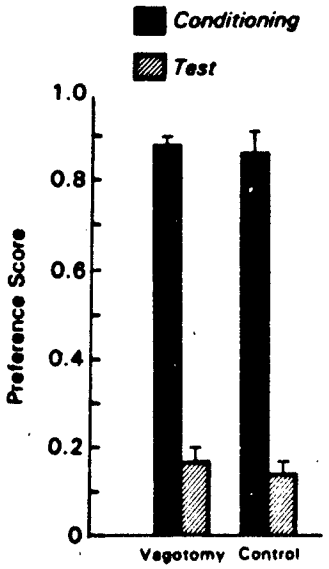

FIG. 1. Effects of subdiaphragmatic vagotomy on sucrose preference following body-only irradiation. Variance bars indicate ± the standard error.

to evaluate the possibility of a neural component involving the vagus nerve, we examined the ability of subdiaphragmatic vagotomies to interfere with the development of a CTA. Subdiaphragmatic vagotomies would disrupt the contribution of vagal input to the area postrema without affecting a humoral mechanism.

Ionizing radiation was used as the toxin in these experiments. Although the effect of subdiaphragmatic vagotomy has been used to study the CTA induced by various drugs without having any significant effect [5, 6, 9, 15], it is worthwhile to use ionizing radiation as a toxin to study the role of vagal involvement in CTA learning for three reasons. (1) Radiation is not a drug circulating in the blood where it could interact directly with the area postrema. (2) The gastrointestinal system is particularly sensitive to damage by ionizing radiation [7]. (3) A CTA can develop after radiation exposure to the abdomen [2]. In order to avoid directly irradiating the area postrema, we shielded the head with lead.

METHOD

Surgery

Male Sprague-Dawley rats (250–350 g) (Charles River) maintained in individual cages in a room with a 12:12, light: dark cycle were used in these experiments. Food (Wayne Lab Blox) and water were freely available, except as otherwise stated. Subdiaphragmatic vagotomies were performed under general anesthesia (120 mg/kg, IP, ketamine and 21 mg/kg, IP, pentobarbital) according to the method of Martin *et al.* [10]. Sham-operated, control animals were handled similarly, except the vagus nerves were not cut. Following surgery all rats were injected with penicillin (30,000 units) and maintained on a diet of wet mash (suspension of crushed laboratory chow in water) for several days before returning to dry pellets. A recovery period of 2–3 weeks elapsed (to avoid possible vagotomy-induced taste aversions [1]) before

TABLE 1
VERIFICATION OF VAGOTOMY

Condition	n	Body Weight (g)	Stomach Weight/ Body Weight Ratio	95% Confidence Limit
Control	11	447 ± 8.9	0.0114 ± 0.0008	0.0158
Vagotomized	11	463 ± 19.4	0.0258 ± 0.0022	

Values are expressed as mean ± the standard error.

the animals were deprived of water (as described below) and before taste aversion training. At the conclusion of the experiments, both control and vagotomized rats were killed with an overdose of pentobarbital (50 mg/kg, IP). Verification of a complete vagotomy was determined both by visual inspection and by comparing the stomach weight/body weight ratios of the vagotomized rats to the controls. Animals with vagotomies have higher ratios than controls because surgery results in reduced stomach emptying time [10].

Taste Aversion Training

The procedures for taste aversion training have been detailed previously [13]. Briefly, the rats were adapted to a water deprivation schedule for 9 days and had access to water for 30 min during the early light phase of the diurnal cycle and again 5 hr later for 15 min. All testing was done during the period in the morning when water was normally available. The procedure was adopted to compensate for possible vagotomy-induced changes in food and water regulation [21]. On the conditioning day (day 10), rats were presented with two calibrated drinking tubes containing a 10% sucrose solution or tap water, and the intake of each fluid was recorded. Immediately after the drinking period, the rats were irradiated. On the test day (day 11), the subjects were again given the two calibrated drinking tubes, and the consumption of each fluid was recorded.

Irradiation

Rats were placed in a plastic restraining tube and received a partial-body dose of 200 rads from a cobalt-60 source at 40 rads/min. Head shielding for body-only exposures was accomplished by enclosing the head of the rat in lead bricks with a minimum thickness of 10 cm. Midlateral doses were determined with a 3.3-cc Victoreen ionization chamber and with thermoluminescent detectors (LiF TLD 100s) at depth in a mouse phantom. Dosimetry indicated that there was less than 1% scatter into the shielded area.

Statistics

Fluid intake is expressed as a preference score, defined as sucrose intake divided by total fluid intake, that is, sucrose intake plus water intake. A preference score greater than 0.5 indicates preference for sucrose, while a score less than 0.5 indicates aversion to sucrose. For statistical analysis, the preference scores were transformed using the arcsin transformation to normalize the distributions [22] and subjected to a 2-way analysis of variance with one repeated measure. Complete vagotomies were presumed when the stomach

weight/body weight ratio was greater than the 95% confidence interval for the controls. Animals that did not meet this criterion were excluded. Only two vagotomized rats did not qualify.

RESULTS AND DISCUSSION

The results of subdiaphragmatic vagotomy on the acquisition of a radiation-induced CTA are found in Fig. 1. As can be seen, the rats acquired a CTA after exposure to radiation. However, statistical analysis indicated that subjecting animals to a bilateral subdiaphragmatic vagotomy did not prevent the acquisition of a CTA following body-only irradiation. The main effect comparing the conditioning day with the test day was significant, $F(1,20)=150.8$, $p<0.001$. However, the main effect for surgery comparing vagotomy to sham-operated controls was not significant, $F(1,20)=0.48$, $p>0.05$, as well as the surgery vs. day interaction, $F(1,20)=0.21$, $p>0.05$. The completeness of the vagotomies was determined from the stomach weight/body weight ratios determined during autopsy. Table 1 illustrates that animals had successful bilateral vagotomies as evidenced by significantly higher ratios than the sham-operated animals based on the 95% confidence interval for the controls.

The data obtained from this study do not support a role of the vagus nerve and, therefore, a major neural component in the acquisition of a radiation-induced CTA. Although negative, these findings are important because they show that a toxin that cannot directly interact with the area postrema can still induce a CTA, even in the absence of a major efferent input from the gut to the area postrema. In addition, they are consistent with other studies in which vagotomy was not effective in reducing CTAs induced by a variety of other toxins, including apomorphine, lithium chloride, ethanol, or copper sulfate [5, 6, 9, 15]. Consequently, the accumulated evidence supports the involvement of a blood-borne, humoral factor, possibly released from injured tissue, in the acquisition of a CTA induced by radiation and other toxins that act through the area postrema.

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